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Review

Cell migration: Rho GTPases lead the way

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Abstract

Rho GTPases control signal transduction pathways that link cell surface receptors to a variety of intracellular responses. They are best known as regulators of the actin cytoskeleton, but in addition they control cell polarity, gene expression, microtubule dynamics and vesicular trafficking. Through these diverse functions, Rho GTPases influence many aspects of cell behavior. This review will focus specifically on their role in cell migration.

Keywords: Cell migration; Rho GTPase; Cell behavior

Introduction

Cell migration is an essential process in all multicellular organisms and is important not only during development, but also throughout life such as in wound repair and during immune surveillance. In the animal, cell migration is directed by extracellular cues acting either as attractants or repellants. These may be soluble factors that can act at a distance, or local signals received from neighboring cells or extracellular matrix. They elicit a large variety of intracellular responses that include changes in the organization of the actin and microtubule cytoskeletons, in vesicular transport pathways and in gene transcription.

Migration mechanisms have been extensively studied in tissue culture, where the environment can be controlled and easily manipulated. In addition, the genetic analysis of whole organisms has made significant and novel contributions (Lehmann, 2001). More recently, the exciting possibility of visualizing migration in the whole animal has become a reality through direct imaging of fluorescently tagged cells (Ahmed et al., 2002). It is now widely accepted that the major driving force of migration is the extension of a leading edge protrusion or lamellipodium, the establishment of new adhesion sites at the front, cell body contraction, and detachment of adhesions at the cell rear (Fig. 1). All these steps involve the assembly, the disassembly or the reorganization of the actin cytoskeleton, and each must be coordinated both in space and time to generate productive, net forward movement.

A huge variety of intracellular signaling molecules have been implicated in cell migration, including MAPK cascades, lipid kinases, phospholipases, Ser/Thr and Tyr kinases and scaffold proteins. However, one particular family of proteins seems to play a pivotal role in regulating the biochemical pathways most relevant to cell migration, the Rho GTPases. This review will focus on the key contributions of Rho GTPases to the process of cell migration. More extensive reviews of Rho GTPases and their roles in related processes, such as neuronal axon extension and growth cone guidance, can be found elsewhere (Grunwald and Klein, 2002; Meyer and Feldman, 2002).

The Rho GTPase switch

Rho GTPases are ubiquitously expressed and 20 members have been identified in mammals, 7 in *Drosophila melanogaster*, 5 in *Caenorhabditis elegans* and 15 in *Dictyostelium discoideum* (Schultz et al., 1998). They act as molecular switches to control signal transduction pathways by cycling between a GDP-bound, inactive form and a GTP-bound,

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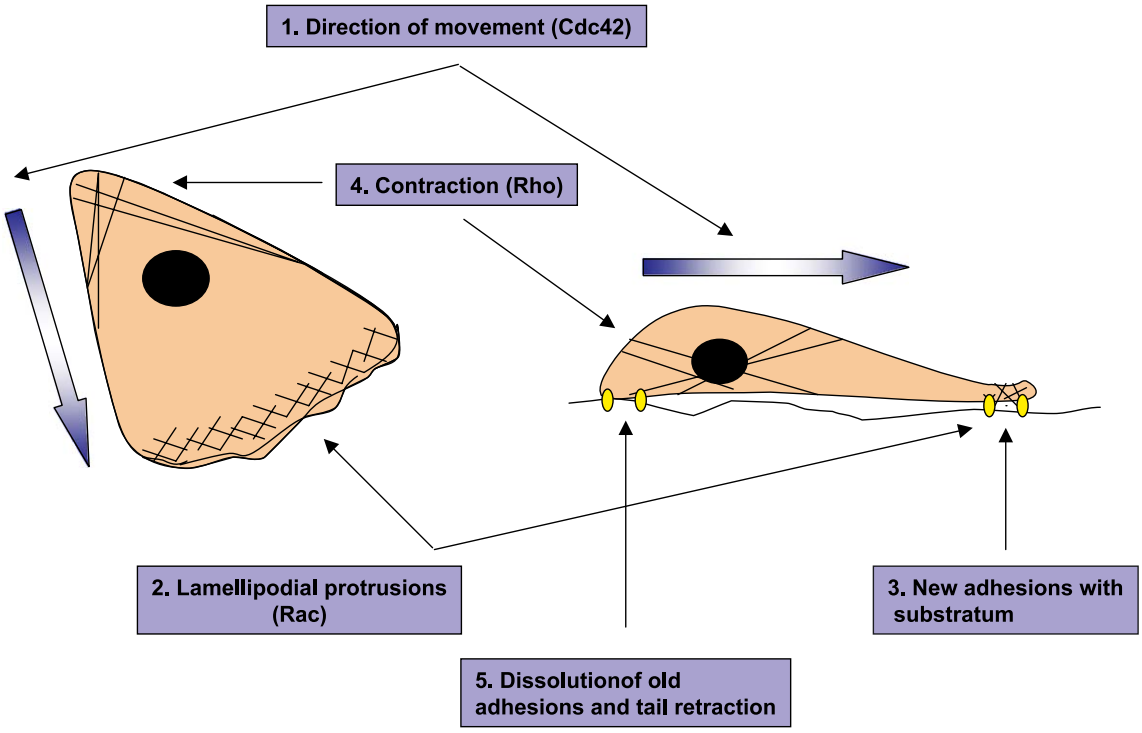

Fig. 1. A migrating cell (seen from the top and side). A migrating cell needs to perform a coordinated series of steps to move. Cdc42 regulates the direction of migration, Rac induces membrane protrusion at the front of the cell through stimulation of actin polymerization and integrin adhesion complexes, and Rho promotes actin:myosin contraction in the cell body and at the rear.

active form. In their GTP-bound state, they interact with downstream targets (effectors) to elicit a variety of intracellular responses. Their best-characterized function is in the regulation of actin dynamics. Tissue culture studies (carried out originally in fibroblasts, but later in many other cell types) using constitutively active and dominant negative, interfering forms, have shown that Rho regulates the assembly of contractile, actin:myosin filaments, while Rac and Cdc42 regulate the polymerization of actin to form peripheral lamellipodial and filopodial protrusions, respectively. In addition, all three GTPases promote the assembly of integrin-based, matrix adhesion complexes (Nobes et al., 1995; Ridley and Hall, 1992; Ridley et al., 1992). It is perhaps not surprising, therefore, that these three regulatory proteins play such an important part in controlling cell migration. Furthermore, and in addition to their effects on actin, Rho, Rac and Cdc42 can influence a wide range of other biochemical activities. Most notably, Cdc42 is required for the establishment of cell polarity, while all three can, in distinct ways, affect the microtubule cytoskeleton and gene transcription (Etienne-Manneville and Hall, 2002).

The Rho GTPase cycle is tightly regulated by three groups of proteins (Fig. 2). Guanine nucleotide exchange factors (GEFs) promote the exchange of GDP for GTP to activate the GTPase, GTPase-activating proteins (GAPs) negatively regulate the switch by enhancing its intrinsic GTPase activity and guanine nucleotide dissociation inhibitors (GDIs) are thought to block the GTPase cycle by sequestering and solubilizing the GDP-bound form (Moon and Zheng, 2003; Schmidt and Hall, 2002; Zheng, 2001). Extracellular signals could regulate the switch by modifying any of these proteins, but so far at least, they appear to act predominantly through GEFs. Once activated, Rho GTPases interact with cellular target proteins (effectors) to generate a downstream response (Bishop and Hall, 2000). To date, more than 40 effectors, 50 GEFs and 40 GAPs have been described for the mammalian Rho family. Since this area is too complex to cover in this short review, we will focus on pathways that have been specifically linked to cell migration.

Regulation of Rho GTPases during cell migration

Rac is required at the front of the cell to regulate actin polymerization and membrane protrusion. For efficient cell migration, this activity would be expected to be spatially restricted and new fluorescent imaging techniques are beginning to reveal that this is indeed the case. Using the FLAIR technique (fluorescence activation indicator for Rho proteins), for example, a gradient of active Rac can be visualized in migrating fibroblasts with the highest concentrations at the leading edge (Kraynov et al., 2000). Rho, on the other hand, is thought to regulate the contraction and retraction forces required in the cell body and at the rear, and would be expected to follow an inverse distribution to Rac, though this

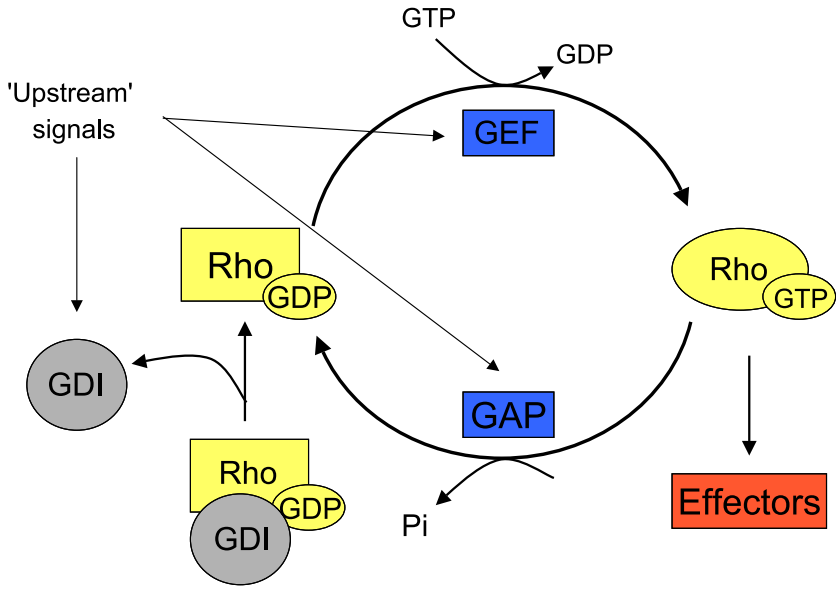

Fig. 2. The Rho GTPase cycle. Rho GTPases cycle between an inactive GDP-bound form and an active GTP-bound form. The cycle is tightly regulated mainly by guanine exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine dissociation inhibitors (GDIs). In their active form, Rho GTPases can bind to effector molecules such as kinases and scaffold proteins.

has not yet been directly demonstrated. The mechanisms regulating Rho activity during cell migration have not been well studied to date. Much more effort has been focused on mechanisms of Rac activation and two pathways have emerged as being particularly important and widespread.

PI 3-kinase and PI(3,4,5)P_3

PI 3-kinases and their lipid product PI(3,4,5)P_3 have been widely implicated in controlling cell migration and polarity (Stephens et al., 2002). During leukocyte chemotaxis, type IA PI 3-kinases are required for lamellipodium extension and migration towards colony-stimulating factor 1 (CSF-1) (Vanhaesebroeck et al., 1999), whereas type IB PI 3-kinases are required for neutrophil chemotaxis to a variety of inflammatory mediators that signal via G-protein-coupled receptors (Hirsch et al., 2000; Li et al., 2000; Sasaki et al., 2000). GFP-tagged pleckstrin homology (PH) domains have been used to visualize bursts of PI(3,4,5)P_3 induced in response to extracellular stimuli, and studies reported in *Dictyostelium*, neutrophils and fibroblasts reveal colocalization with centers of actin polymerization at the front of the migrating cell (Haugh et al., 2000; Parent et al., 1998; Servant et al., 2000). Elegant studies in *Dicytostelium* have shown that the localization of PI 3-kinase at the front is coupled to a restricted distribution of its antagonist, the PI(3,4,5)P_3 phosphatase PTEN, at the rear, and that this is essential for chemotaxis (Funamoto et al., 2002; Iijima and Devreotes, 2002).

The production of PI(3,4,5)P_3 leads to an increase in GTP-bound Rac in many cell types. Expression of a constitutively activated PI 3-kinase in fibroblasts, for example, generates extensive lamellipodia and membrane ruffling through Rac activation, though interestingly not other Rac-mediated signals such as JNK activation (Reif et al., 1996). The mechanism by which this lipid promotes GTP loading on Rac is thought to be through a direct interaction with Rac GEFs (Fig. 3). All members of the Dbl family of GEFs contain a PH domain and at least some of these can bind phospholipids. In Vav2, for example, PI(3,4,5)P_3 binding relieves an interaction between the PH and DH (catalytic) domains to stimulate activity (Abe et al., 2000; Han et al., 1998), though this is unlikely to be the whole story since tyrosine phosphorylation also occurs (Liu and Burridge, 2000; Sachdev et al., 2002). Deletion of the PH domain in the GEFs Dbs and Lfc, on the other hand, results in a loss of activity, but this can be restored by addition of a CAAX motif to target the protein to the plasma membrane (Whitehead et al., 1995, 1999). In this case, a major role of PI(3,4,5)P_3 is thought to be in inducing membrane translocation. Tiam-1, a Rac specific GEF, provides another example where the PH domain regulates targeting to the plasma membrane as it can be functionally replaced by a myristoylation signal (Michiels et al., 1997). Finally, PIX is regulated by PI(3,4,5)P_3. When localized at the front of a migrating cell, PIX activates Rac and promotes actin polymerization, but it also interacts with the Rac target p65PAK and is thought to regulate focal adhesion turnover (Bagrodia and Cerione, 1999; Koh et al., 2001; Manser et al., 1998; Yoshii et al., 1999).

The relationship between Rac and PI 3-kinase during cell migration may be more interesting, since the two are able to interact directly with each other and Rac activation stimulates PI 3-kinase leading to the production of PI(3,4,5)P_3 (Benard et al., 1999; Bokoch et al., 1996; Genot et al., 2000; Hawkins et al., 1995; Servant et al., 2000; Zheng et al., 1994). This would provide an opportunity for a positive feedback loop (Fig. 3). To explore this possibil-

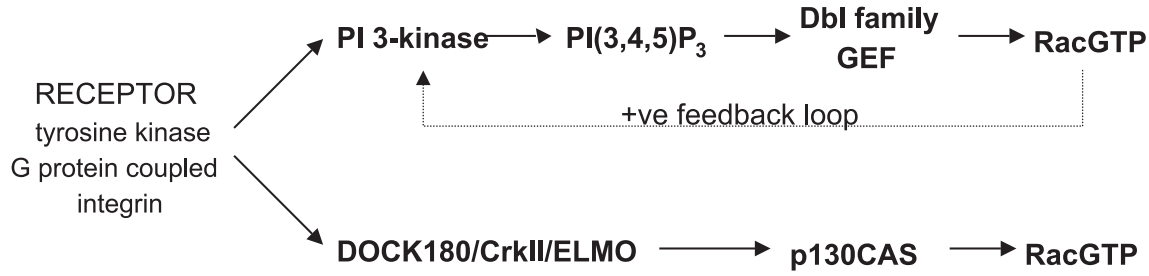

Fig. 3. Activation of Rac during cell migration. Two important pathways have emerged that activate Rac downstream of membrane receptors during cell migration. Many DH-containing GEFs that are active on Rac are regulated by the lipid PIP_3, and in some cases, Rac then acts in a positive feedback loop by activating PI 3-kinase. A novel GEF, DOCK180, is thought to act in a complex with three other proteins (CrkII, ELMO and p130CAS) to promote activation of Rac. Genetic and biochemical experiments have revealed an important role for this mechanism of Rac activation during cell migration. The significance of these two apparently quite distinct mechanisms of Rac activation is unclear.

ity, Bourne and coworkers made use of neutrophils expressing the PH domain of Akt coupled to fluorescent green protein (PH-Akt-GFP) as a probe for the spatial distribution of $PI(3,4,5)P_3$ (Wang et al., 2002; Weiner et al., 2002). They found that in response to a chemoattractant or an exogenously delivered bolus of $PI(3,4,5)P_3$, neutrophils asymmetrically accumulate $PI(3,4,5)P_3$ and filamentous actin at the leading edge, and this involves a positive feedback loop operating between PI 3-kinase and Rac. Disruption of this feedback loop results in a jerky, non-polarized cell response to chemoattractants. The exchange factors regulating Rac during neutrophil chemotaxis are not known, although the RacGEF PREX-1 has recently been shown to be activated by $PI(3,4,5)P_3$ in neutrophils leading to Rac-dependent stimulation of the NADPH oxidase (Welch et al., 2002).

The p130Cas/CrkII/DOCK180 pathway

DOCK180 was originally identified as a binding partner of the SH2/SH3-containing adaptor protein Crk (Buday et al., 2002; Hasegawa et al., 1996). Crk in turn associates with another adaptor molecule p130Cas and Crk/p130Cas expression can lead to Rac activation and promote cell migration (Klemke et al., 1998) (Fig. 3). Expression of DOCK180 has been shown to potentiate p130Cas/Crk-induced cell migration, which can be blocked by inhibition of Rac (Cheresh et al., 1999).

Understanding the function of DOCK180 has been greatly facilitated by genetic studies in flies and worms. DOCK180 and its orthologs, *mbc* (myoblast city) in *Drosophila* and *ced-5* in *C. elegans*, belong to an evolutionarily conserved family of proteins, termed CDM (Wu and Horvitz, 1998) that are involved in a variety of developmental processes linked to Rac function. Mutations in *mbc*, for example, result in defects in myoblast fusion, dorsal closure and migration of epidermal cells reminiscent of a loss of Drac1 (*Drosophila* Rac1) phenotype (for a recent review on dorsal closure, see Jacinto et al., 2002). Mbc is also required for the Rac-dependent border cell migration during oocyte maturation in response to EGF and PDGF stimulation (Duchek and Rorth, 2001; Duchek et al., 2001).

Ced-5 was originally identified along with six other *ced* genes as being needed for the engulfment of apoptotic cells during *C. elegans* development (Ellis et al., 1991). It was noticed then that Ced-5, Ced-2 and Ced-10 were each required for migration of the distal tip cells (DCTs) of the gonads (Wu and Horvitz, 1998). Interestingly, Ced-2 and Ced-10 encode the worm orthologs of CrkII and p130CAS, respectively. Further genetic analysis has identified another protein, Ced-12 (mammalian ortholog ELMO1), which regulates the Ced-5 (DOCK180) complex. Ced-12/ELMO is an evolutionarily conserved protein and is involved in phagocytosis, cell migration and cell shape changes at a step upstream of Ced-10 and Rac by forming a complex with Ced-2/CrkII and Ced-5/DOCK180 (Gumienny et al., 2001; Wu et al., 2001; Zhou et al., 2001) (Fig. 3). More recently, studies in knockout mice have revealed a role for the hematopoietic-cell-specific homologue of DOCK180, DOCK2, in lymphocyte chemotaxis. $DOCK2^{-/-}$ T and B lymphocytes are significantly impaired in their ability to migrate to the lymph nodes or the spleen compared to $DOCK2^{+/-}$ cells when challenged with the chemokine SDF-1 (Fukui et al., 2001; Reif and Cyster, 2002). These and other studies have clearly demonstrated a role for the CDM family members in regulating a variety of Rac-dependent processes including apoptosis, cell migration and axonal outgrowth, which are induced by integrin, growth factor or chemokine receptors (Albert et al., 2000; Wu et al., 2002).

The biochemical mechanism by which the p130Cas/Crk/DOCK180 complex activates Rac is becoming clearer. DOCK180/ELMO1 immunoprecipitates contain a RacGEF activity and DOCK180 itself interacts with nucleotide-free Rac, a characteristic of exchange factors. However, neither ELMO1 nor DOCK180 have the conserved Dbl-homology (DH) domain found in all known members of the RhoGEF family (Gumienny et al., 2001; Kiyokawa et al., 1998; Schmidt and Hall, 2002). One possibility is that DOCK180/ELMO recruits a DH-containing GEF to the complex, which carries out Rac exchange. In the case of lymphocytes, one

such GEF could be Vav, which coprecipitates with DOCK2. However, dominant negative Vav does not block activation of Rac by DOCK2 (Nishihara et al., 2002).

Two independent studies have now shed new light on this problem and identified an evolutionarily conserved domain, Docker/DHR-2, in the C-terminus of DOCK180 that can stimulate GTP loading on Rac (Brugnera et al., 2002; Cote and Vuori, 2002). One group has suggested that in vivo, ELMO is essential for exchange activity and that a DOCK180/ELMO complex acts as a bipartite, unconventional GEF. Another group has, however, reported that the Docker/DHR-2 domain is sufficient to stimulate the loading of Rac with GTP in vitro and in vivo. Interestingly, another DHR-2-containing protein, DOCK9/zizimin, specifically binds to and activates Cdc42 (Cote and Vuori, 2002; Meller et al., 2002).

Downstream effects of Rho GTPases during cell migration

Regulation of the actin cytoskeleton

Rac and Cdc42 are both required at the front of migrating cells. The primary role of Rac is to generate a protrusive force through the localized polymerization of actin. Cdc42 also induces actin polymerization to generate filopodia often seen at the front of migrating cells (Nobes and Hall, 1995). The role of filopodia is not entirely clear; it is thought that they probe the extracellular milieu, but in many cases they do not seem to be required for migration per se. Recent studies in *Drosophila*, for example, have shown that Cdc42 loss of function mutations do not affect the migration of peripheral glial cells (Sepp and Auld, 2003). Cdc42 does, however, play a crucial role at the front of cells in controlling the direction of migration.

The cellular targets of Rac and Cdc42 that promote changes to the actin cytoskeleton have been the subject of intense investigation. The Ser/Thr kinase p65PAK is commonly activated upon either Rac or Cdc42 activation and is believed to play an important role in regulating actin dynamics and cell adhesion during migration. Efficient cell migration requires integrin-dependent matrix adhesion to generate traction forces at the front and in the cell body, but integrin adhesion complexes must be dynamic and disassembled at the cell rear to allow the cell to pass. p65PAK regulates focal adhesion turnover, with the help of PIX and GIT1 (GRK interactor 1), but how it does so is not known (Manabe Ri et al., 2002; Obermeier et al., 1998). In addition, p65PAK phosphorylates and activates LIM kinase (LIMK), which in turn phosphorylates and inactivates cofilin (Arber et al., 1998; Edwards et al., 1999) (Fig. 4). Cofilin facilitates subunit dissociation from the pointed end of actin filaments and induces filament severing and is essential for promoting filament treadmilling at the front of migrating cells. Its ability to cycle between active (nonphosphorylated) and inactive (phosphorylated) forms is a critical feature of its mechanism of action (reviewed in Bamburg and Wiggan, 2002).

Members of the WASp/SCAR/WAVE family of scaffold proteins are key regulators of actin polymerization (Takenawa and Miki, 2001). In their activated state, each of these proteins is able to stimulate the Arp2/3 complex, which can initiate actin polymerization either de novo or at the barbed end or sides of preexisting filaments. In this way, the dendritic morphology of lamellipodial actin is generated (Weaver et al., 2003) (Fig. 4). WASp/WAVE can also bind to profilin, which acts synergistically with Arp2/3 to speed-up actin polymerization (Blanchoin et al., 2000; Yang et al., 2000). Cdc42 activates WASp and N-WASp directly, although the lipid $PI(4,5)P_2$ is an essential cofactor (Rohatgi et al., 1999, 2000). Rac activates the Scar/WAVE family indirectly and this involves an Nck-adaptor complex (Eden et al., 2002; Rohatgi et al., 2001).

Rho activity in migrating cells is associated with focal adhesion assembly and cell contractility and is responsible for cell body contraction and rear end retraction. One important Rho target involved in stimulating actin:myosin filament assembly and therefore contractility is the Ser/Thr kinase p160ROCK (Fig. 4). Rho and p160ROCK have been shown to be essential for rear cell detachment in single migrating cells such as leukocytes and macrophages, though it may be less important in cells moving together as sheets (Alblas et al., 2001; Nobes and Hall, 1999). p160ROCK plays an essential role during migration of P cells in the larval development of *C. elegans* and during dorsal closure and gastrulation in *Drosophila* (Barrett et al., 1997; Magie et al., 1999; Spencer et al., 2001). In its active state, p160ROCK, like p65PAK, can phosphorylate and activate LIMK, which in turn phosphorylates and inactivates cofilin leading to stabilization of actin filaments within actin:myosin filament bundles (Maekawa et al., 1999; Sumi et al., 2001). p160ROCK interacts with and phosphorylates the myosin binding subunit (MBS) of myosin light chain phosphatase and thereby inactivates it (Kawano et al., 1999). This leads to increased levels of myosin phosphorylation, which then can cross-link actin filaments and generate contractile force (Fig. 4). At the rear of a migrating cell, this promotes movement of the cell body and facilitates detachment of the cell rear (Mitchison and Cramer, 1996).

Clearly, Rho activity at the front of a migrating cell is incompatible with membrane protrusion and hence mechanisms must be in place to inhibit its activity at the leading edge. One way this might occur is through Rac. Expression of activated Rac has been shown to inhibit Rho function in many cell types, ranging from fibroblasts to neurons (Sander et al., 1999). The biochemical mechanisms involved are not entirely clear; one suggestion is that p65PAK phosphorylates and inactivates myosin light chain kinase (MLCK), leading to decreased levels of myosin phosphorylation (Kiosses et al., 1999; Sanders et al., 1999). However, others

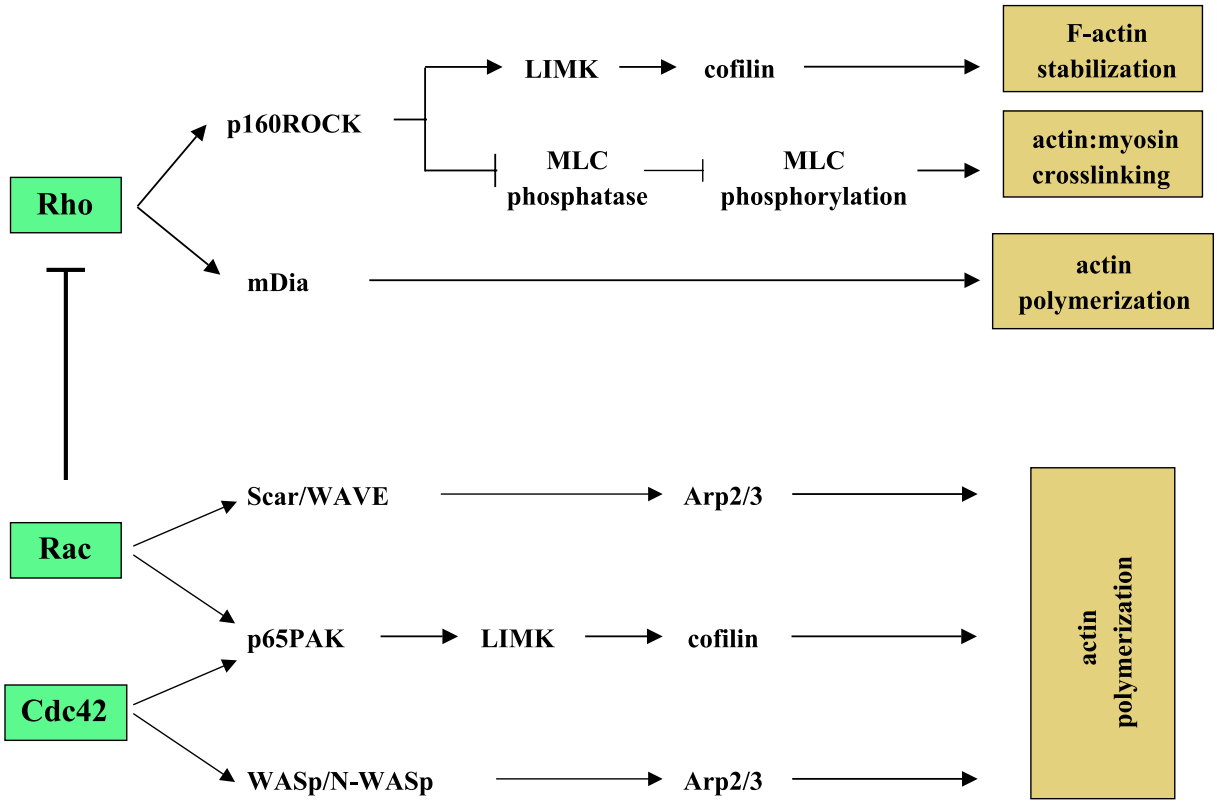

Fig. 4. Rho-GTPase-regulated pathways affect actin filament organization. Rho promotes contractile actin:myosin filament assembly through two effectors, mDia and p160ROCK. Little is known about which molecules lie downstream of mDia, but p160ROCK phosphorylates LIM kinase, leading to cofilin phosphorylation, and myosin light chain (MLC) phosphatase, leading to an increase in MLC phosphorylation. Rac and Cdc42 both regulate actin polymerization through the WASp/Scar/WAVE family of proteins acting on the Arp2/3 complex, and through p65PAK kinase acting on LIM kinase.

have reported that p65PAK can phosphorylate myosin light chain directly, thus enhancing cell contractility (Sells et al., 1999).

Another important downstream target of Rho is mDia, the mammalian ortholog of *Drosophila* Diaphanous. mDia belongs to the formin-homology containing family of proteins, which have been linked to actin filament assembly in both *Drosophila* and yeast (Castrillon and Wasserman, 1994; Pruyne et al., 2002; Sagot et al., 2002). The binding of Rho.GTP to mDia opens up and activates this scaffold protein. It cooperates with p160ROCK in the assembly of actin:myosin filaments, but its biochemical mechanism of action is still unclear (Uehata et al., 1997; Watanabe et al., 1999).

The microtubule cytoskeleton and polarity

Although the effects of Rho GTPases on the actin cytoskeleton have received most attention to date, it is now clear that they can also modulate the microtubule cytoskeleton (Wittmann and Waterman-Storer, 2001). While it is unlikely that the microtubule cytoskeleton plays an essential role during cell migration or chemotaxis over short distances, efficient and persistent long-range migration requires stabilization of cell polarity and this is achieved through reorganization of the microtubule cytoskeleton.

The first clue for a link between Rho GTPases and microtubules was the observation that nocodazole (an inhibitor that disrupts microtubules) activates Rho, but when washed out of cells it leads to Rac activation (Liu et al., 1998). Later, Rho was shown to promote the stabilization of microtubules through its target mDia that directly interacts with microtubules and promotes their capping (Ishizaki et al., 2001; Palazzo et al., 2001a). Rac, on the other hand, may promote microtubule elongation through p65PAK-dependent phosphorylation and inactivation of the microtubule destabilizing protein, stathmin (Daub et al., 2001; Kuntziger et al., 2001) (Fig. 5).

Cdc42 plays a crucial role in defining cell polarity with respect to the external environment. Inhibition of Cdc42 in macrophage cells blocks their ability to undergo chemotaxis towards a gradient of CSF-1, although it does not inhibit their ability to move (which is Rac-dependent) (Ridley, 2001). The mechanism by which Cdc42 regulates chemotaxis is not known. However, work with neutrophils suggests that there exists a positive feedback loop between Rac and PI(3,4,5)P_3 required to establish cellular asymmetry and polarity, and it seems likely that Cdc42 organizes these activities with respect to the external chemotactic gradient (Weiner et al.,

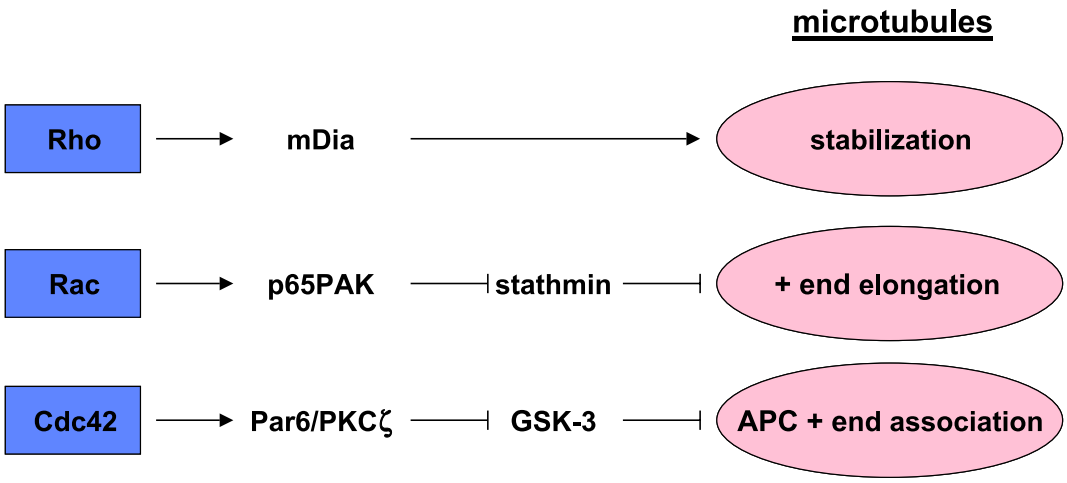

Fig. 5. Rho GTPases regulate microtubules. Rho activation leads to microtubule stabilization through an unknown mechanism involving mDia. Rac activates p65PAK to phosphorylate and inactivate stathmin, a microtubule-destabilizing protein. Cdc42 regulates microtubule and centrosome polarity through activation of the Par6/PKCζ complex, which in turn leads to the inhibition of GSK-3 and the association of APC with the plus end of microtubules.

2002). The polarized migration of many cells is reflected in the reorganization of the microtubule cytoskeleton and the centrosome, which usually (though not always) face the direction of migration. This facilitates cell migration by directing transport pathways to the leading edge and is particularly important to achieve efficient and persistent migration over longer distances (Ma and Chisholm, 2002). In migrating astrocytes and fibroblasts, Cdc42 regulates this reorientation of microtubules and the centrosome and recent work has revealed some of the mechanisms involved (Etienne-Manneville and Hall, 2001; Nobes and Hall, 1999; Palazzo et al., 2001b). In its GTP-bound state, Cdc42 activates a target complex of Par6 (a scaffold protein) and the atypical protein kinase C, PKCζ (Etienne-Manneville and Hall, 2001). The specific activation of this complex at the leading edge of migrating cells is essential for determining the orientation of the cell during migration. Further work on astrocytes has uncovered more of the signaling pathway downstream of Cdc42 (Etienne-Manneville and Hall, 2003). PKCζ phosphorylates and inactivates GSK-3 and this induces the association of APC with the plus ends of microtubules specifically at the leading edge. Through a dynein- or dynactin-dependent mechanism, this then results in microtubule reorganization and centrosome reorientation (Fig. 5). Whether part or all of this pathway controls chemotaxis downstream of Cdc42 in macrophages or neutrophils remains to be seen.

Interestingly, APC interacts with Asef, a Rac-specifc GEF, pointing to possible cooperation between Rac and Cdc42 at the leading edge (Kawasaki et al., 2000). There is also evidence that APC can move along microtubules, probably through binding to the microtubule-associated protein EB1 and the kinesin/KAP3 motor complex (Gundersen, 2002; Jimbo et al., 2002; Nakamura et al., 2001). The movement of APC towards the plus ends of microtubules in migrating cells could serve to localize Asef to sites of Rac-dependent actin polymerization (Bienz, 2002; Jimbo et al., 2002).

Conclusions

For cell migration to occur, there is a constant need for the cell to coordinate a variety of intracellular activities both spatially and temporally. Rho GTPases are key players in this process and their ability to cycle between active and inactive states allows the cell to respond rapidly to extracellular cues. The challenge for the future is to understand how the cell compartmentalizes, yet cooperatively couples, Rho and Rac activities needed to drive the cell forward. Superimposed on this machinery is a still poorly understood process by which Cdc42 "samples" the external environment and controls directionality.

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